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(57) Abstract

A synthetic carrier for delivering at least one biologically active component to an organism utilizing a synthetically assembled peptide which may be linked to a resin. The carrier peptide includes a terminal amino acid having a pair of functional end sites, which are capable of bonding at least a pair of components including biologically active components. Further, additional amino acids may be attached to the branched terminal amino acid chain to form a matrix having a progressively larger number of branches and attachment sites. Biologically active components may be attached to the multiple branch chains with a high degree accuracy. Moreover, additional intermediate attachment sites of the matrix may be used to link other functional groups such as adjuvant peptides. The synthetic branched chain carrier may be employed to construct a synthetic immunogen of a very high purity and possessing specific coupling ratios among the various components attached to the ends of the branched chains and at other pre-defined intermediate attachment sites. Thus, specific combinations of biologically active components may be delivered by the synthetic carrier of the present invention.

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SYNTHETIC CARRIER AND IMMUNOGEN

BACKGROUND OF THE INVENTION

The present invention relates to a novel synthetic carrier, used for constructing a biologically active component delivery vehicle such as an immunogen utilizing the same.

Vaccines have been developed to inoculate animals against virulent diseases such as poliomyelitis, measles, small pox, and the like. Such immunization has been effected by utilizing an attenuated or killed virus in the animal to elicit antibodies which kill the disease organism. Unfortunately, attenuated viruses have an uncertain level of danger in the animal injected with the same. In addition, the vaccines utilizing killed or attenuated viruses are easily contaminated and require special handling such as refrigeration prior to use.

Synthetic vaccines have attracted great interest since they do not employ killed or attenuated viruses. Rather, peptides are used to mimic portions of a particular virus which elicit the antibodies. This effect was described by Richard A. Lerner in an article entitled "Synthetic Vaccines", Scientific American, Pages 66-74, February 1983.

Utilization for biological treatment of various types of low molecular weight compounds such as peptides, nucleotides, steroids, toxins, carbohydrates, and other small organic compounds for synthetic immunogens is known. These low molecular weight compounds have either been isolated from natural sources or produced synthetically. In addition, these low molecular weight compounds have been attached to a carrier molecule such as a protein. Specifically, the carriers used have been keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin, bovine thyroglobulin, and the like. Such low molecular weight compound-protein conjugates are required to elicit an antibody response in an animal. It is well documented that small molecules are not recognized by the immune system. Thus, there has been a problem in the past to

increase the size of the small molecules such that the immune system will recognize the low molecular weight component of the immunogen. It is believed that the animal's immune system recognizes the high molecular weight conjugate, while multiple copies of the small molecular weight compound attached to the "processed fragments" of the protein conjugate elicit an antibody response.

In the case of a low molecular weight antigen, simply constructing large molecular weight protein conjugates does not guarantee the eliciting of a strong antibody response in an animal. It is well documented that an immunomodulator, known as an adjuvant, is required for strong antibody responses. Various types of materials ar known to function as adjuvants. The two most widely used are an alum precipitate and Freund's complete adjuvant (FCA). The former is a colloidal suspension of a water soluble protein or low molecular weight compound-protein conjugate combined with water insoluble aluminium hydroxide. FCA is a mixture of water immiscible mineral oils containing heat killed tuberculosis bacteria. Typically on the research level, the antibody response which is elicited using FCA is the standard, against which all other responses are evaluated and judged. Ellouz et al in an article in Biochem. Biophys. Res. Commun., 59, (1979) page 1317, investigated the adjuvant effect produced by FCA. It was concluded that this effect was due to a small fragment of the cell wall peptidoglycan of the heat-killed tuberculosis bacterium. This cell wall fragment, N-acetyl-muramyl-L-alaninyl-D-isoglutamine, is called muramyl dipeptide (MDP) or more commonly, adjuvant peptide. J. Tam in an article in Proc. Natl. Acad. Sci. U.S.A., 85, (1988) page 5409, described a method for building multiple copies of a synthetic peptide on a functionalized branched chain core attached to a solid support resin. This process called Multiple Antigenic Peptide (MAP) synthesis produces multiple copies of a low molecular weight peptide on a branched chain core. The net effect of MAP is to produce

a molecule with the molecular weight of a small protein. For example, a 15 amino acid long peptide with a molecular weight of approximately 1,600 Daltons when built on a 16 branched chain core of Lys_8-Lys_4-Lys_2-Lys-X-X-Resin would have a molecular weight of about 27,000 Daltons. Although useful as an antigen for the production of antibodies specific to the synthetic peptide, the MAP system only performs this function. In another words, the MAP approach eliminates the need for a high molecular weight carrier protein, but does not deliver additional biologically active components such as adjuvants, cytotoxins, fatty acids, and the like. In addition, the MAP approach suffers from numerous problems. First and most importantly, during the solid phase synthesis of the MAP, the peptide antigens are synthesized directly on the branched chain core. This does not allow for purification of the synthetic peptide. Thus, the peptide antigen component of the MAP is actually a complex mixture of numerous peptides of unknown and undefined sequence. In fact, various investigators hav reported on the elicitation of a strong antibody response to minor impurities in preparations of synthetic peptides. It has been concluded that the only way to avoid this problem is to used highly purified synthetic peptides of known structure. MAP is incapable of solving this problem. Further, MAP does not allow for the construction of antigens with other non-peptide components such as carbohydrates, glycopeptides, glycoproteins, nucleotides, nucleopeptides, peptides, or proteins built by other methods such as solution phase synthesis or by genetic engineering. In addition, structurally complex peptides are not capable of being synthesized as a MAP structure. For example, peptides with disulfide bonds, which are the best analogues of whole protein loop structures, li in this category.

United States Patent 5,198,531 to Webber et al describes a notable advance in the preparation of peptides utilizing a polymeric resin. The resin of the 5,198,531

patent permits the building of peptides of exceptional purity, greatly eliminating undesirable side reactions during the process.

A synthetic carrier for delivering biologically active components such as antigens, cytotoxins, drugs, adjuvants, fatty acids, alone and/or in combination, at a specific level or ratio, would be a major advance in the medical and biological fields.

SUMMARY OF THE INVENTION

In accordance with the present invention a novel and useful carrier for biologically active components to an organism is herein provided.

The present invention comprises a synthetic branched chain carrier for delivering at least on biologically active component to an organism. The carrier employs a first unbranched peptide chain which may or may not be linked to a solid support resin. The first peptid may be formed of an unbranched peptide segment or analogue of any length and include amino acids therealong that are capable of linking biologically active components, to their individual sidechains. In addition, the unbranched peptide segment terminates in an amino acid having a pair of end sites, thus providing a branched site. The second peptide is attached to one of the pair of end sites of the first peptide, while a third peptide is attached to the other of said pair of end sites of the first peptide. The second and third peptides may be identical and each include a terminal amino acid having a pair of end sites capable of bonding a pair of biologically active components. Bonding is used to include covalent and ionic bonding, electrostatic interaction, hydrophobic interaction, Vander Waals force attraction, and the like. Thus, a quartet of end sites are available in the basic synthetic carrier of the present invention, as well as a pre-determined number of intermediate sites found along the unbranched segments of the first, second, and/or third peptide, at individual side chains.

The biologically active components may take the form of immunogenic peptides, adjuvants, fatty acids or alcohols, cytotoxins, drugs, nucleotides and the like. Those components may be placed at the end sites or intermediate sites in specific numbers and, thus, in specific ratios when more than one component is employ d. In this regard, a plurality of components may be used in the carrier of the present invention, each having a

specific biological function. For example the biologically active component may be a receptor peptide coupled with a drug, cytotoxin, or the like.

The carrier of the present invention may be expanded to multiple levels of peptide segments, having branched end sites, connected to the pair of end sites of each amino acid terminating each peptide. That is to say, at each successive branched level, the number of end sites would double from the prior level. Thus, at the fifth level, utilizing the first peptide as the (zero) level, (32) end sites would be available for combining biologically reactive components.

A particular use of the carrier of the present invention is to form an immunogen which will elicit an immunological response in an animal. Such immunogen may be considered as a vaccine in certain cases when the response concerns viral entities.

It may be apparent that a novel and useful synthetic carrier for delivering a biologically active component to an organism has been described.

It is therefore an object of the present invention to provide a synthetic carrier for delivering at least one biologically active component which is capable of delivering such component in a pre-defined amount to an organism.

Another object of the present invention is to provide a synthetic carrier which is capable of delivering a multiplicity of biologically active components in pre-defined ratios.

A further object of the present invention is to provide a synthetic carrier for delivering at least one biologically active component to an organism which may be an immunogen.

Yet another object of the present invention is to provide a synthetic carrier for delivering biologically active components to achieve a variety of results such as drug treatment, delivering a cytotoxin to a specific site,

and the like.

A further object of the present invention is to provide a synthetic carrier for delivering a biologically active component which activates major histocompatibility complexes I, II, and/or III.

Yet another object of the present is to provide a synthetic carrier which is useful in the production of a synthetic vaccine.

The invention possesses other objects and advantages especially as concerns particular characteristics and features thereof which will becom apparent as the specification continues.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a depiction of the overall matrix of the carrier of the present invention taken from levels "0" through "5", still attached to a solid support resin n which it was synthesized.

Fig. 2 is a formulation of a component bound to an intermediate site of the matrix of Fig. 1.

Fig. 3 is a depiction of MDP bound to ornithine at an intermediate site of the matrix of Fig. 1.

Fig 4 is a depiction of a fatty alcohol bound to a site of the matrix of Fig. 1.

Fig. 5 is a depiction of a fatty acid bound to a site of the matrix of Fig. 1.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Various aspects of the present invention will evolve from the following detailed description of the preferred embodiments, which should be taken in conjunction with the prior described drawings.

The synthetic branched chain carrier peptide of the present invention may take the following general formulation as attached to the solid phase support resin:

Formula 1

Resin-PPP-QQQ-RRR-Lys-$(SSS-TTT-Lys)_2$-$(UUU-VVV-Lys)_4$-$(WWW-XXX-Lys)_8$-$(YYY-ZZZ-Lys)_{16}$

Where PPP, QQQ, SSS, TTT, UUU, VVV, WWW, XXX, YYY, ZZZ, represent amino acid. Or other components built into the carrier peptide structure, which function either as "spacers", functional "attachment sites" or for other purpose. Lys represents a dibasic amino acid such as lysine, ornithine, 2, 3-diaminopropionic acid, 2, 4-diaminobutyric acid and the like. Such dibasic amino acids can serve as branch points and attachment sites. With reference to Fig. 1, it may be observed that a branched carrier may be built under the present invention to extend to various levels. Fig. 1 shows levels 0-5 where the fifth level furnishes (32) end attachment sites capable of attaching biologically active components such as an antigen, cytotoxin, drug, monoclonal antibody, and the like. The carrier depicted in Fig. 1 and described above may be built following the procedures described in United States Patent 5,198,531.

Fig. 2 represents a typical functional group or component designated "Com" attached to intermediate functional group site 12, denoted VVV at level 2 of Fig. 1. In addition, "Com" is possible in the present invention by designing in appropriate functional groups prior to the provision of the branched end chain 14. Functional groups at site 12 can take the form of trifunctional amino acids like aspartic acid or glutamic acid, both of which have a -COOH functional group on their respective side chains. In

addition, cysteine may be employed to provide a -SH functional group on its side chain. Moreover, lysine may be employed in this regard utilizing its epsilon-NH_2 group. It should be noted that each of these functional groups may be differentially protected. Moreover, other groups may be used to incorporate more specialized components. In addition, it is possible to incorporate the same functional group at the particular level shown in Fig. 1 prior to a branch point and to protect, differentially, the same to allow different components to be attached thereto. Thus, cysteine may function as an attachment site for more than one type of biologically active molecule to be delivered to an organism. Fig. 3 depicts the attachment of muramyl dipeptide (MDP) to ornithine. MDP is an adjuvant peptide which would be attached through routine chemistry, known in the peptide building art.

It should be particularly noted that the placement of different biologically active components at the (32) available sites on the fifth level of the carri r of Fig. 1 and to intermediate sites, such as site 12, is practicable. Moreover, biological active components may be placed at these sites in the carrier of the present invention in specific numbers and in various coupling ratios. It is also practical to provide more complex biologically active components for specialized applications. Specifically, three or more components may be added to the carrier of Fig. 1 at the (32) available end sites of the fifth level, and at intermediate level sites 12 and 16. As an example, a kidney receptor peptide may be placed on the (32) available end sites at the fifth level while a drug may be attached to the intermediate site 12. Further, a tumor receptor peptide may be placed at the (32) available end sites while a cytotoxin is placed at intermediate site 12 or 16. It should be stressed that the position of the biologically active components may be reversed i.e. a drug may be placed on the (32) available end sites while a receptor peptide may be placed on any of

the intermediate sites, or built into the carrier as the initial unbranched peptide or peptide analogue segment.

With reference to Fig. 4, attention is drawn to site 18 at level "0" where a fatty alcohol may be attached to the carboxyl terminal amino acid after it has been cleaved from the resin. This may be accomplished by addition of the fatty alcohol to the free -COOH end of the amino acid PPP following cleavage from the resin. Fig. 5 illustrates that a fatty acid may be attached to the trifunctional group designated Lys after cleavage of the carrier peptide at group "PPP", from the resin. This can be achieved by protecting the epsilon $-NH_2$ group of Lys and treating the same with TFA. The fatty acid would normally be pre-activated, with hydroxybenzotriazole and diisopropylcarbodiimide (HOBt+DIPCDI), for example. Attachment of the fatty alcohol or fatty acid as noted in Fig. 4 and 5 may be accomplished by other known chemical processes. Thus, it is possible with the carrier of the present invention to bond a fatty acid or fatty alcohol as an anchor for the carrier of the present invention to liposomes, micelles, or other hydrophobic structures. The fatty acid or alcohol anchor may be combined with a targeting receptor peptide and/or a drug attached to either the (32) end sites of Fig. 1 or an intermediate site such as site 12 or 16 of Fig. 1. Again, such a trio of components may be placed on th carrier of the present invention in specific ratios. It should be observed, that the branched configuration of Fig. 1 may be lengthened or shortened such that the carrier includes sites at the first level only or includes sites beyond the fifth level. Although the carrier of the present invention may be used for other purposes than to build a synthetic immunogen, this particular application is especially useful. Specifically, disulfide cyclized peptide analogues of the CD_4 binding site of the HIV-1 virus may be synthesized and used in conjunction with the carrier of the present invention. Other synthetic immunogens may

be constructed using other peptide analogues to various viral components.

While, in the foregoing, embodiments of the present invention have been set forth in considerable detail for the purposes of making a complete disclosure of the invention, it may be apparent to those of skill in th art that numerous changes may be made in such detail without departing from the spirit and principles of the invention.

The present invention is illustrated by the following examples, but is not deemed to be restricted thereby. Abbreviations appearing therein are ones normally used in the fields of immunology and solid phase peptide synthesis. The one letter abbreviations appearing in peptide chains represent standard amino acid denotations.

EXAMPLE 1

A first peptide, (identified as "PC-1580") was synthesized at the 0.05 mmole scale by standard Fmoc-Solid Phase Peptide Synthesis (Fmoc-SPPS) using hydroxybenzotriazole/diisopropyl carbodiimide (HOBt/DIPCDI) procedures and a four molar excess of reagents on a Dasrin-3 resin available from Research and Diagnostic Antibodies, Berkeley, California. The sequence of the (40) amino acid long peptide was as follows:

NH_2-LPCRIKQIINMWQEVGKAMYAPPIEGQIR-ßAla-ßAla-GGEFFYCNS-COOH

Once completed, the peptide was cleaved from the resin and the side chains of the trifunctional amino acids were simultaneously deprotected using 40 ml of 50% trifluoroacetic acid (TFA) in dichloromethane (DMC) containing 1ml H_2O, 0.5 ml thioanisole, 210 mg dithiothreitol (DTT) and 38 1 anisole. The spent resin was washed with 100% acetic acid (HOAc), 50% HOAc/50% water, and water. The solutions were pooled, rotary evaporated twice, and the peptide partially purified by gel filtration chromatography on a 70 ml column of Sephad x G-10 , washed and equilibrated with 25% HOAc in water. The column was developed with 25% HOAc in water and fractions

were collected. The fractions which contained the peptide were identified by optical density (OD) at 270nm and by a qualitative spot test developed with ninhydrin. Fractions #7-10 were found to contain the peptide. These fractions were pooled, the total volume adjusted to 800 ml with water, the acetic acid neutralized, and the pH adjusted to pH 8.1 with dilute NH_4OH. The peptide was air oxidized f r three days with constant slow mixing to form the intrachain disulfide bond and cyclize the peptide. The loss of free -SH groups was monitored during the air oxidation by th quantitative DTNB assay. The solution was centrifuged to remove precipitated material, and pumped at 3 ml/min onto a semi-preparative C_{18} reverse phase HPLC column. The peptide was eluted from the column with a gradient of acetonitrile. The eluent from the column was monitored for OD at 286nm and fractions were collected. The fractions which contained peptide were transferred to pre-weighed vials, lyophilized, and analyzed. Fractions #16 and 17 were pooled and yielded 22 mg of peptide at greater than 99% purity. Based upon a molecular weight of 4531.4, starting with 0.05 mmole of derivatized resin, and obtaining 22 mg of finished product, the overall yield for this synthesis, cyclization, and purification procedure was calculated to be 9.7%.

EXAMPLE 2

A second peptide (identified as PC-1581) was synthesized by Fmoc-SPPS using (4) molar excess of reagents, cleaved, isolated, cyclized, purified, and analyzed by the same procedures as described above for th peptide, (PC-1580), of Example 1.

The sequence of this (39) amino acid long peptide was as follows.

NH_2-NCGGEFFY-βAla-βAla-RLKQIINMWQEVGKAMYAPPIEGQIRCSS-COOH

The overall process yielded 26 mg of peptide at gr ater than 96% purity which, based upon a molecular weight f 4408.2 and a 0.05 mmole scale synthesis, represents an

11.8% yield.

EXAMPLE 3

A branched chain synthetic carrier peptide of the type depicted in Fig. 1 was synthesized and conjugated with muramyl dipeptide, adjuvant peptide. The carrier sequence was as follows:

$$AGAGK-(GK)_2-(GK[boc]GK)_4-(GK)_8-(GGK[difmoc])_{16}$$

where the subscript numbers represent the number of terminal lysines at each level, shown in Fig. 1. Th branched chained synthetic carrier peptide was synthesized employing the DASRIN-3 resin of Example 1 using the Fmoc-SPPS/HOBt/DIPCDI strategy. The reagents were used at a four fold molar excess at each coupling step, and each coupling reaction was incubated overnight with constant mixing. The initial starting scale of the synthesis was 0.01 mmole at level zero which then became a 0.16 mmole scale synthesis during the level four coupling cycles due to the doubling which occurred at each branch point. Four Lys(boc) residues were inserted in intermediate level (2) as attachment sites for adjuvant peptide. The fmoc protecting groups were initially left on the completed peptide in order to block the 16 terminal lysines' (32) $-NH_2$ groups from reacting with the adjuvant peptide during its attachment to the internal or intermediate lysines on level (2). The peptide was cleaved from the resin, and the boc protecting groups on the internal lysines wer simultaneously removed using 40 ml of 25% TFA in DCM which contained 1.0 ml of water. The spent resin was washed with 25% TFA in DCM and then with DCM. The solutions were pooled and rotary evaporated three times. The residual TFA was neutralized with diisopropylethylamine (DIEA), and then the branched chain peptide was reacted with 0.16 mmole of MDP which is a four fold molar excess over the fre internal lysine attachment sites on level (2). Following attachment of the four adjuvant peptides per molecule, th (32) fmoc groups on the terminal (16) lysines were removed with 20% pyrrolidine in DCM. The solvents were removed by

rotary evaporation three tim s. The material was diluted with DCM, neutralized with HOAc, and extracted twice with water to separate the peptide from the residual organics. Initial purification of the muramyl dipeptide$_4$/branched chain synthetic carrier peptide conjugate was achieved by gel filtration on a 180 ml column of Sephadex G-10 which was washed and equilibrated with 0.1 M ammonium bicarbonate pH 8.0. The fractions were analyzed. Those found to contain the conjugate (MDP$_4$/Carrier) were pooled (fractions #9-14), rotary evaporated twice, and purified by reverse phase HPLC on a semi-preparative scale C_{18} column. The fractions found to contain the peptide were lyophilized individually in pre-weighed vials for analysis. Fractions #7 - 9 were pooled to yield 39 mgm of conjugate at greater than 97% purity. Based upon a molecular weight of 9540.5 for the conjugate and upon a starting scale synthesis of 0.01 mmole, the overall yield was 40.9%.

EXAMPLE 4

Two totally synthetic immunogens were built using the PC-1580 of Example 1 and MDP$_4$/Carrier of Example 3, and using PC-1581 of Example 2 and MDP$_4$/Carrier of Example 3. Each molecule of MDP$_4$/Carrier had a total of (32) free NH_2s as the termini of the (16) branches of the carrier peptide. A three fold molar excess of HOBt/DIPCDI activated synthetic antigenic peptide, either PC-1580 or PC-1581 was used. 10 mg of PC-1580 was activated with a molar equivalent of HOBt and DIPCDI. The activated antigenic peptide was then reacted with 0.22 mg (23.0 nmole) of MDP$_4$/Carrier overnight in DMF with constant mixing. The reactions was stopped by the addition of water which hydrolyzed the activated esters, thus allowing for the excess antigenic peptide to be recovered for reutilization. The PC-1580$_{32}$/MDP$_4$/Carrier synthetic immunogen (calculated Mol. Wt. 154,532) was isolated from the free antigenic peptide, PC-1580 (Mol. Wt. 4531) and low molecular weight organic molecules by gel filtration on Sephadex G-25 in 0.1 M acetic acid. The synthetic immunogen eluted in the void

volume of the column. These fractions were pooled and lyophilized. The fractions containing the free peptide were also collected and lyophilized for reuse. In a similar manner, 10 mg of PC-1581 was reacted with 0.23 mg of MDP_4/Carrier to build PC-1581_{32}/MDP_4/Carrier, synthetic immunogen. The overall yields for these two reaction sequences were 3.3 mg (92.9% yield) and 3.0 mg (84.3% yield) for PC-1580 and PC-1581, respectively. Both of the totally synthetic immunogens were then used to immunize animals.

EXAMPLE 5

Both the PC-1580 and PC-1581 of Example 1 and 2, respectively, were conjugated onto bovine thyroglobulin using EDAC as the cross linking reagent. The peptides wer activated with EDAC and then conjugated to the protein in a two step reaction sequence. The coupling ratios used were 200 molecules of antigenic peptide per molecule of carrier thyroglobulin protein. Each reaction was perform d using standard procedures and routine reaction conditions. The conjugates were isolated from the reaction mixture by gel filtration on Sephadex G-25, dialyzed, and lyophilized. The yields based upon the weights of the final products were greater than 95% for both the PC-1580/thyroglobulin and PC-1581/thyroglobulin conjugates. Both of the peptide/protein conjugates were then used in conjunction with Freund's complete adjuvant to immunize animals.

EXAMPLE 6

Four groups of three rabbits each were immunized with one of the four different immunogens: either 1. PC-1580_{32}/MDP_4/Carrier in saline, 2. PC-1581_{32}/MDP_4/Carrier in saline, 3. PC-1580/thyroglobulin in an oil in water emulsion with Freund's complete adjuvant, or 4. PC-1581/thyroglobulin in an oil in water emulsion with Freund's complete adjuvant per Examples 1-5. All animals followed the same boost/bleed schedule which is detailed b low.

DAY	PROCEDURE

0 Immunize intramuscularly in the left thigh
7 Immunize intramuscularly in the right thigh
56 Boost subcutaneously at multiple sites on the back
70 Bleed from the central ear artery
Continue 14 day/14 days boost/bleed cycles

ELISA was used to assess the response each of th immunogens had in eliciting the production of antibodies in each animals of all the test groups. The ELISA conditions used for these assays are as follows.

A. Sensitize plates overnight with either 100 ng of antigenic peptide per well, with 40 ng of MDP_4/Carrier per well, or with 40 ngm of bovine thyroglobulin per well in bicarbonate buffer pH 9.6
B. Wash x 2 with PBS/tween
C. Block with 0.1% BSA in PBS
D. Wash x 2 with PBS/tween
E. Two fold serial dilutions of antisera bound overnight
F. Wash x 4 with PBS/tween.
G. Affinity purified HRP-goat anti-rabbit IgG-2nd antibody bound for 3 hrs
H. Wash x 4 with PBS/tween
I. OPD with H_2O_2 reaction run for 30 min and stopped with sulfuric acid
J. Read plates at 492 nm

The assays were set up on 96 well high binding microtiter plates as follows:

Row A = Serial dilutions of pre-immune serum from one animal
Row B - D = Serial dilutions of the antisera obtained from one animal of the group
Row E = Serial dilutions of the pre-immun serum from another animal
Row F - H = Serial dilutions of the antisera obtained

from another animal of the group

Column 1 = Blank (No serum bound to well)

Columns 2-12 = two fold serial dilutions of antiserum from

1:1000 to 1:1,024,000

The titers, which are based upon half-maximal OD readings, are tabulated below both for response to the antigenic peptide and for response to the carrier for each animal of the four groups.

Table 1

ELISA Results for PC-1580_{32}/MDP_4/Carrier

Animal #	Bleed #	Titer for Peptide	Titer for Carrier
R-2161	1st	20,000	< 1000
	2nd	25,000	< 1000
	3rd	60,000	< 1000
R-2162	1st	200,000	< 1000
	2nd	400,000	< 1000
	3rd	500,000	< 1000
R-2163	1st	< 1000	< 1000
	2nd	5,000	< 1000
	3rd	25,000	< 1000

Table 2

ELISA Results for PC-1581_{32}/MDP_4/Carrier

Animal #	Bleed #	Titer for Peptide	Titer for Carrier
R-2164	1st	90,000	< 1000
	2nd	150,000	< 1000
	3rd	120,000	< 1000
R-2165	1st	250,000	< 1000
	2nd	90,000	< 1000
	3rd	80,000	< 1000
R-2166	1st	400,000	< 1000
	2nd	500,000	< 1000
	3rd	300,000	< 1000

Table 3

ELISA Results for PC-1580/Thyroglobulin with FCA

Titer	Titer

Animal #	Bleed #	for Peptide	for Carrier
R-2167	1st	< 1000	40,000
	2nd	2,000	150,000
	3rd	8,000	400,000
R-2168	1st	4,000	100,000
	2nd	12,000	250,000
	3rd	25,000	300,000
R-2169	1st	6,000	100,000
	2nd	10,000	120,000
	3rd	12,000	150,000

Table 4

ELISA Results for PC-1581/Thyroglobulin with FCA

Animal #	Bleed #	Titer for Peptide	Titer for Carrier
R-2170	1st	30,000	80,000
	2nd	25,000	150,000
	3rd	25,000	400,000
R-2171	1st	10,000	150,000
	2nd	6,000	250,000
	3rd	12,000	300,000
R-2172	1st	2,000	400,000
	2nd	8,000	700,000
	3rd	10,000	>1000,000

EXAMPLE 7

One antiserum from each of the four groups f rabbits of Example 6 was selected for use in west rn immunoblot and IFA experiments. Whole cell lysates fr m cells which had been transfected with the HIV-1 genome and which have been shown to produce various HIV-1 proteins were subjected to SDS-PAGE following standard procedures. After electrophoresis, the proteins were electrophoritically transferred to PVDF membranes and the membranes were blocked with non-fat milk. Alternating lanes on the membrane had either diluted primary antibody or diluted primary antibody that had been blocked by preincubation for 60 min. with 100 gm/ml of antigenic peptide, either PC-1580 or PC-1581. Both the primary and

blocked primary antibody were allowed to bind for (2) hours. The membrane strips were washed twice before affinity, purified HRP-goat anti-rabbit IgG second antibody was then bound for 60 minutes. The membranes were washed four times. The DAP/H_2O_2 color reaction was performed. Each of the four antisera bound specifically to two bands on the membranes. These proteins had molecular weights of 120 kD and 160 kD, respectively, and were identified as HIV-1 gp120 and gp160. No bands were detected on the strips to which the peptide blocked antibodies were applied. It is believed that this example demonstrates that the anti-peptide antibodies elicited by the synthetic immunogen as-well-as those elicited by the carrier protein can recognize and bind specifically to whole proteins which contain an analogous amino acid sequence to that of the antigenic peptide.

EXAMPLE 8

The antibodies of Example 6 were used in indirect immunofluorescent assay (IFA) staining experiments on fixed whole cells which produce HIV-1 gp120 and gp160 in cultur . In these IFA experiments the antibodies have been found to bind specifically to HIV-1 transfected cells. They do not, however, bind to non-transfected normal cells, and the binding can be blocked by pre-incubating the dilut d primary antibody with antigen peptide before it is applied to the fixed cells. The proteins in this Example had not been denatured as they were in the western immunoblots following SDS-PAGE, in Example 7. Again, it is believed this Example demonstrates that the antibodies elicited by the totally synthetic immunogen of the present invention can recognize and bind to whole proteins which have amino acid sequences analogous to that of the antigenic peptide incorporated into the synthetic immunogen of the present invention.

WHAT IS CLAIMED IS

1. A carrier for delivering at least on biologically active component to an organism,

comprising:

a. a first peptide, said first peptide including a terminal amino acid having a pair of ends capable of serving as branch sites;

b. a second peptide attached to one of said pair of end sites of said first peptide; and

c. a third peptide attached to one of said pair of end sites of said first peptide, said second and third peptides each including a terminal amino acid having a pair of end sites capable of bonding a pair of biologically active components.

2. The carrier of claim 1 in which each terminal amino acid of said second and third peptides is a dibasic amino acid.

3. The carrier of claim 2 in which said terminal dibasic amino acid is selected from the group consisting of:

lysine, ornithine, 2,3-diamino/propionic acid, and 2,4-diaminobutyric acid.

4. The carrier of claim 1 which additionally comprises at least a pair of biologically active components bound to each end of said terminal amino acid.

5. The carrier of claim 4 in which each of said components bonded to said terminal amino acid is a peptid .

6. The carrier of claim 5 in which said peptide terminates in a pair of functional ends.

7. The carrier of claim 1 in which said first, second, and third peptides further selectively include an intermediate amino acid having a side chain capable of bonding to a biologically active component.

8. The carrier of claim 4 in which said pair of components bonded to each chain of said terminal amino acid of said second and third peptides is a cytotoxin.

9. The carrier of claim 4 in which said pair of

components bonded to each chain of said terminal amino acid of said second and third peptides is a monoclonal antibody.

10. The carrier of claim 4 in which said pair of components bonded to each chain of said terminal amino acid of said second and third peptides is a drug.

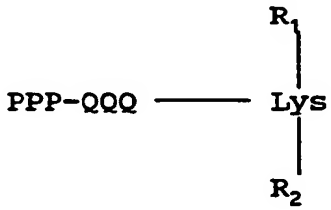
11. The carrier of claim 7 in which said peptide includes a first biologically reactive component bonded t said end chain of said terminal amino acid and a second biologically effective group bound to said side chain of said intermediate amino acid.

12. The carrier of claim 1 in which said second and third peptides are identical.

13. A carrier for delivering at least n biologically reactive component to an organism,

comprising:

a peptide having the formula:



wherein PPP, and QQQ represents at least one amino acid, R_1 and R_2 represent a biologically active component, and Lys represents an amino acid having a pair of end sites.

14. The carrier of claim 13 in which R. represents a peptide terminating in an amino acid having branched attachment sites for biologically active components.

15. The carrier of claim 13 wherein PPP represents an amino acid having a side chain available for attaching of biologically active component.

16. The carrier of claim 13 wherein QQQ represents an amino acid having a side chain available for attaching a biologically active component.

17. The carrier of claim 16 wher in PPP represents an amino acid having a side chain available for

attaching a biologically active component.

18. The carrier of claim 14 in which PPP represents an amino acid having a side chain available for attaching a biologically active component.

19. The carrier of claim 18 in which QQQ represents an amino acid having a side chain available for attaching a biologically reactive component.

20. The carrier of claim 18 in which PPP represents an amino acid having a side chain available for attaching a biologically active component.

21. The carrier of claim 15 which additionally composes a biologically reactive component attached to said side chain of said amino acid designated PPP.

22. The carrier of claim 21 in which said biologically active component is a fatty alcohol.

23. The carrier of claim 21 in which said biologically reactive component is a fatty acid.

24. The carrier of claim 16 which additionally comprise a biologically active component attached to said side chain of said amino acid designated QQQ.

25. The carrier of claim 24 in which said biologically active component is an adjuvant.

26. The carrier of claim 24 in which said biologically active component is a drug.

27. The carrier of claim 24 in which said biologically active component is a cytotoxin.

28. The carrier of claim 24 in which said biologically reactive component is a peptide.

29. The carrier of claim 24 in which said biologically reactive components is a protein.

30. The carrier of claim 13 in which said PPP component is linked to a polymeric resin.

31. The carrier of claim 13 in which said PPP component is a nucleotide.

32. The carrier of claim 13 in which said PPP component is an amino acid analogue.

33. The carrier of claim 13 in which PPP

possesses a free carboxyl group capable of attaching a biologically active component selected from the group consisting of: a fatty acid, a fatty alcohol, a nucleotide, a drug, and a cytotoxin.

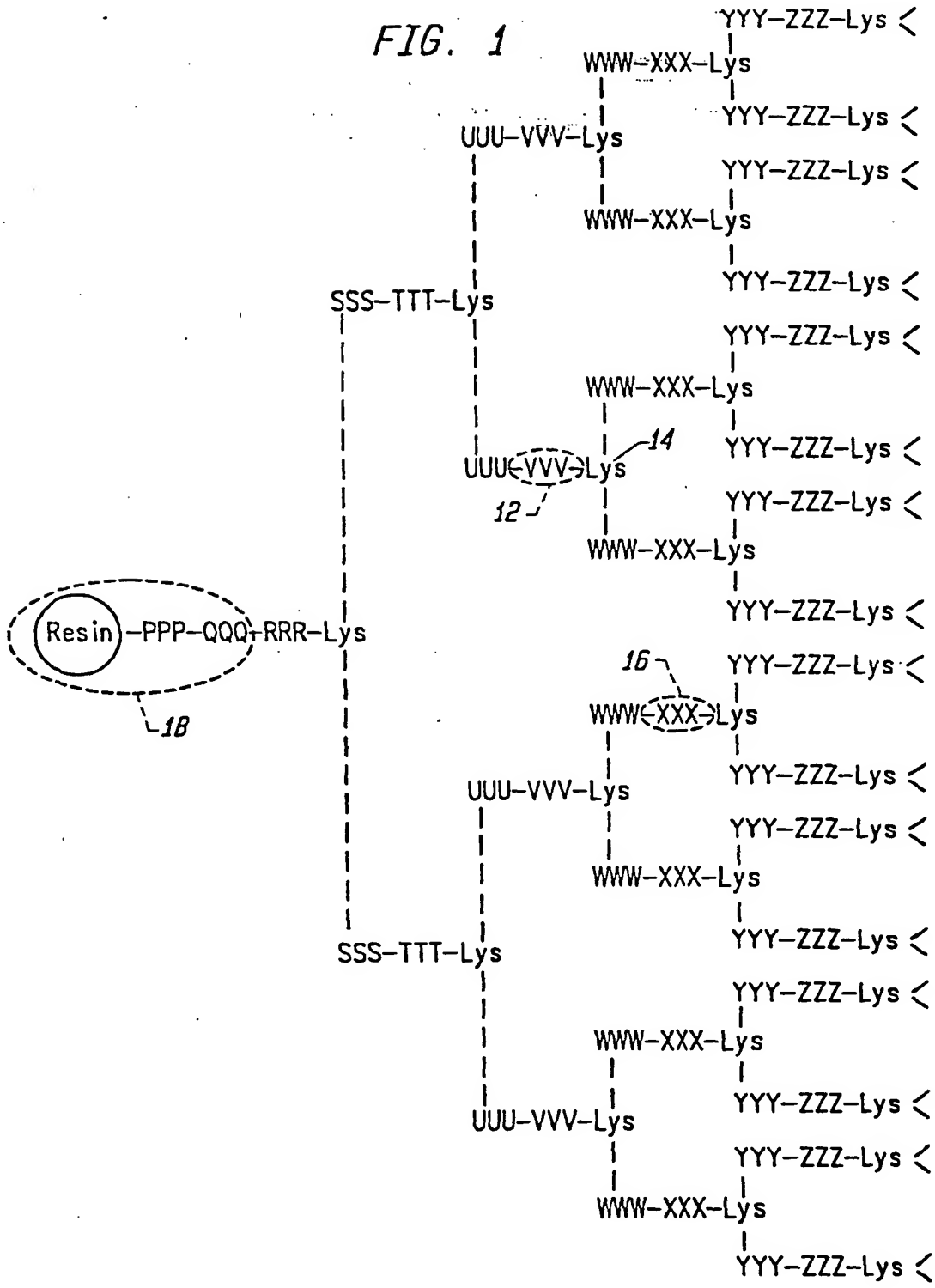
34. A carrier for delivering at least one biologically reactive components to an organism.

comprising:

a peptide of a pre-determined length including a terminal amino acid having a branched chain, said chain being capable of binding at least one pair of biologically reactive components, said peptide further including an intermediate amino acid possessing a side chain capable of binding at least another biologically reactive components at said terminal amino acid.

35. The carrier as shown by the formulation of Fig. 1.

FIG. 1


Resin
PPP
QQQ
RRR
Lys
SSS
TTT
UUU
VVV
WWW
XXX
YYY
ZZZ
12
14
16
18
LEVEL:
ZERO
1ST
2ND
3RD
4TH
5TH
NUMBER OF POSSIBLE ATTACHMENT SITES PER COMPONENT ON EACH LEVEL:
$2^0=1$
$2^1=2$
$2^2=4$
$2^3=8$
$2^4=16$
$2^5=32$

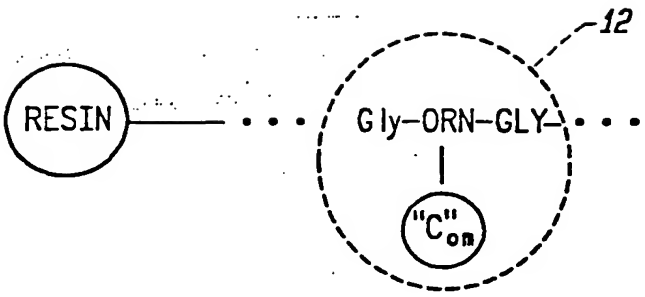


FIG. 2

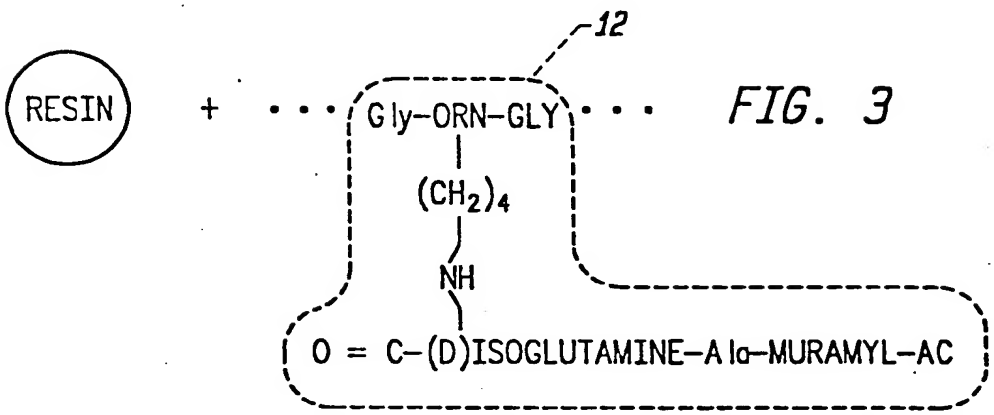


FIG. 3

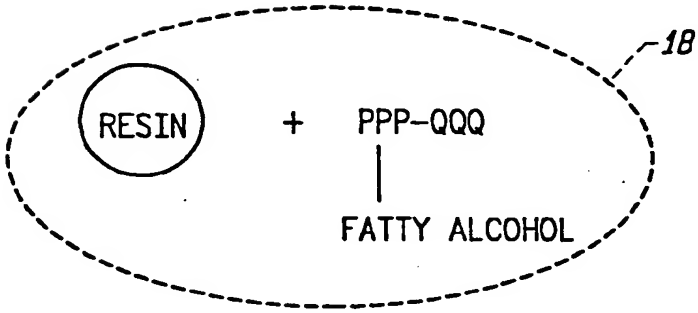


FIG. 4

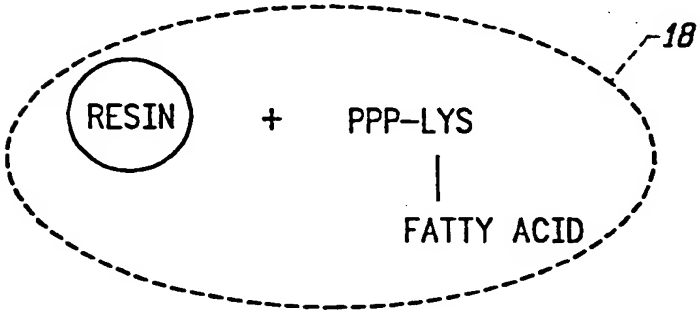


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05981

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K-7/02, 15/28, 17/02
US CL :530/323; 332; 391.1, 402
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/323, 332, 391.1, 402; 424/178.1; 184.1, 193.1; 436/544; 435/961

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FILE CA, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Angew. Chem. Int. Ed. Engl., Volume 29, issued 1990, D. A. Tomalia et al, "Starburst Dendrimers: Molecular-Level Control of Size, Shape, Surface Chemistry, Topology, and Flexibility from Atoms to Macroscopic Matter", pages 138-175, see page 167.	1 - 7 , 1 1 - 21,24,28,30,32-35 1-35
X Y	EP, A, 0 339 695 (BLOEMHOFF ET AL) 02 November 1989, see abstract and Figure 1.	1-7,11-21,24,28,30,32-35 1-35
X Y	EP, A, 0 343 460 (SINIGAGLIA) 29 November 1989, see abstract and Figure 2.	1 - 7 , 1 1 - 21,24,28,30,32-35 1-35

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 SEPTEMBER 1994	Date of mailing of the international search report SEP 19 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KAY K. KIM, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05981

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Bioconjugate Chemistry, Volume 1, No. 5, issued September/October 1990, J. C. Roberts et al, "Using Starburst Dendrimers as Linker Molecules to Radiolabel Antibodies", pages 305-308, see the entire article.	1-35
X	GB, B, 2 228 262 (TALWAR ET AL) 22 August 1990, see pages 6-7.	1,4-7,10,13-27,32,34,35
X Y	US, A, 4,289,872 (DENKEWALTER ET AL) 15 September 1981, columns 3-4.	1-4,7,8,10-21,24,26,27,32-35 1-35
X Y	J. A. SMITH ET AL eds, "PEPTIDES: Chemistry and Biology, Proceedings of the Twelfth American Peptide Symposium June 16-21, 1991, Cambridge, Massachusetts, USA" published 1992 by ESCOM (Leiden), pages 847-848, see the entire article.	1,4-7,11-21 ,23-26,28,32-35 22
X Y	Int. J. Peptide Protein Res., Volume 40, issued 1992, J. Defoort et al, "A rational design of synthetic peptide vaccine with a built-in adjuvant", pages 214-221, see page 219.	1,4-7,11-21 , 23-26,28,32-35 22
X Y	R. EPTON ed, "INNOVATION AND PERSPECTIVES IN SOLID PHASE SYNTHESIS: Peptides, Polypeptides and Oligonucleotides", published 1992 by Intercept Limited (Andover), pages 241-249, see pages 242 and 247.	1,4-7,11-21,23-26,28,32-35 22